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Review

# Innate Immune Sensing of HIV-1 by Dendritic Cells

Jeremy Luban[1,2,*]
[1]Department of Microbiology and Molecular Medicine, University of Geneva, 1211 Geneva, Switzerland
[2]Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA 01605, USA
*Correspondence: jeremy.luban@umassmed.edu
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**HIV-1-specific antibodies and CD8$^+$ cytotoxic T cells are detected in most HIV-1-infected people, yet HIV-1 infection is not eradicated. Contributing to the failure to mount a sterilizing immune response may be the inability of antigen-presenting dendritic cells (DCs) to sense HIV-1 during acute infection, and thus the inability to effectively prime naive, HIV-1-specific T cells. Recent findings related to DC-expressed innate immune factors including SAMHD1, TREX1, and TRIM5 provide a molecular basis for understanding why DCs fail to adequately sense invasion by this deadly pathogen and suggest experimental approaches to improve T cell priming to HIV-1 in prophylactic vaccination protocols.**

Currently, more than 30 drug formulations are approved that block HIV-1 replication and prevent infected individuals from progressing to AIDS (http://www.fda.gov). However, treatment with these drugs is lifelong and plagued with toxicity, virus-drug resistance, and significant economic cost. According to recent figures from UNAIDS, 34 million people are infected with HIV-1, and for each person who starts anti-HIV-1 drug treatment, it is estimated that there are 2–3 new infections. In 2010, 1.8 million people died of AIDS-related illnesses and 2.6 million became infected with HIV-1. Efforts to control the AIDS pandemic would benefit from an effective anti-HIV-1 vaccine, but with perhaps one exception (Rerks-Ngarm et al., 2009), attempts to prevent new HIV-1 infection in human vaccine trials have been unsuccessful.

The eradication of smallpox and the effective control of poliovirus, measles, mumps, rubella, and yellow fever offer stark contrast to the public health experience with HIV-1. The live virus preparations used to immunize against these pathogens were developed empirically, without understanding the mechanisms that underlie the anamnestic response. The success of the vaccines against these viruses—particularly those vaccines that replicate within the host—demonstrates that lifelong protective immune responses can be elicited by vaccination.

In contradistinction, people infected with HIV-1 progress to AIDS despite measurable humoral and cellular immune responses to HIV-1 (Virgin and Walker, 2010). Worse still, HIV-1-infected people with documented, broad anti-HIV-1 immune responses can be secondarily infected with HIV-1 (Altfeld et al., 2002; Smith et al., 2005). Interestingly, failure to protect against reinfection is also seen with Hepatitis C virus (Blackard and Sherman, 2007), and no vaccine is available for this virus either. These observations do not mean that an HIV-1 prophylactic vaccine is impossible, especially given that superinfection with HIV-1 might be aided by the immune dysfunction associated with prior HIV-1 infection. Nonetheless, these observations demonstrate that the immune response targeting HIV-1 differs fundamentally from that against the viruses described above and suggest that, in the absence of some fundamental modification in vaccine design, even a live vaccine would be unlikely to alter the outcome of an HIV-1 challenge.

While failure to elicit protective immunity distinguishes HIV-1 (and HCV) from pathogenic viruses such as poliovirus and measles, these are not the only viruses that have eluded efforts to develop a protective vaccine. Dengue infects 500 million people each year, two million of whom suffer complications of hemorrhagic fever (Beatty et al., 2010). Like HIV-1, there are multiple types of Dengue virus, there is no good animal model, and there are no simple correlates of immunologic protection. The first protective Dengue vaccine—albeit with 30% efficacy—was possible only recently, after 50 years of research (Sabchareon et al., 2012). These results are remarkably similar to the reported 31% efficacy in RV144, the only successful HIV-1 prophylactic vaccine trial (Rerks-Ngarm et al., 2009).

Respiratory Syncytial Virus (RSV) causes >100,000 hospitalizations for pneumonia each year in the United States (Welliver, 2003). As with HIV-1, RSV-specific immune responses are detectable after infection, but protection against recurrent infection is not conferred. Attempts to vaccinate against RSV even caused a paradoxical increase in disease severity, perhaps because the particular adjuvant used—alum—elicited a nonprotective CD4$^+$ $T_H2$ response (Lindell et al., 2011). Ultimately, any advance in mechanistic understanding of protective immunity targeting HIV-1 would likely benefit attempts to control Dengue virus, RSV, and other viruses as well.

This review will assess the large body of literature on HIV-1 and construct a model to explain why the human immune system fails to eliminate or prevent HIV-1 infection. In large part, it will focus on recent developments regarding host cell restriction factors and attempt to link these findings to what is known about innate immune detection of HIV-1, T cell priming by DCs, and HIV-1 vaccine development.

## What Permits HIV-1 to Escape Control by the Human Immune System?

Several hypotheses offer plausible explanations for HIV-1 persistence in the face of apparent antiviral immunity. Soon after establishment of infection by one or a few HIV-1 virions, variants are selected in response to pressure from HIV-1-specific cytotoxic T lymphocytes (CTLs) and neutralizing antibody (Kearney et al., 2009; Henn et al., 2012). It should be noted that high mutagenic rates and complex mixtures of sequence variants known

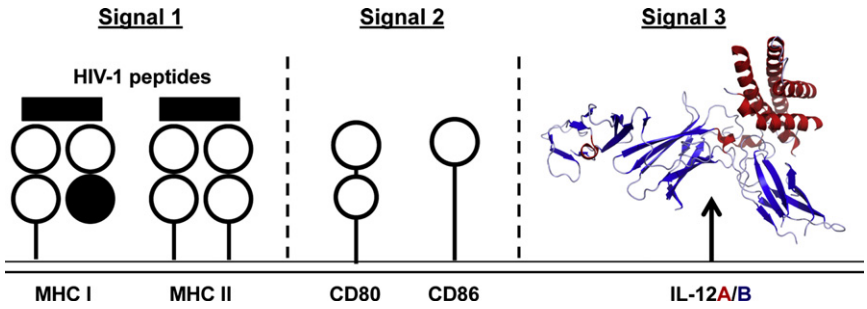


**Figure 1. Dendritic Cells Provide Multiple Signals to Naive T Cells**

The innate immune system instructs the acquired immune system via a diverse set of signals that dendritic cells provide to naive, antigen-specific T cells. A range of outcomes are possible, from tolerance to potent antiviral immunity, depending upon the exact signals provided. Protective antiviral immunity requires that naive T cells specific for viral antigens receive three signals from DCs (Reis e Sousa, 2006). Elaboration of the three signals requires DC maturation, most effectively attained by activation of cell-intrinsic pattern recognition receptors. The first signal involves antigen presentation via MHC. The second signal is provided by cell surface molecules that include CD80 and CD86. The third signal is delivered by cytokines that include IL-12, the structure of which is shown (PDB: 1F45) (Yoon et al., 2000); the two IL-12 subunits are distinguished from each other by red (IL12A, p35) and blue (IL12B, p40).

as quasi-species are also well described for other RNA viruses, including some viruses for which there are effective vaccines. The pathogenesis of poliovirus, for example, requires an error-prone viral polymerase and the quasi-species that it generates (Vignuzzi et al., 2006). This suggests that it is not quasi-species per se that distinguishes HIV-1 from other viruses, but rather HIV-1's capacity to replicate in the face of enormous sequence variation.

The HIV-1 Env glycoprotein, in particular, is distinguished by great sequence diversity and conformational flexibility, as well as an extensive glycan shield and viral entry receptor binding sites that are well concealed and only accessible to antibodies that have undergone extensive somatic mutation (Zhou et al., 2007; Scheid et al., 2009; Bar et al., 2012). Details about this extraordinary molecule and how broadly neutralizing antibodies might be generated by prophylactic vaccination is discussed in an accompanying review by Dennis Burton and colleagues (Burton et al., 2012).

Another intuitive explanation for the inability of the immune system to clear infection is that HIV-1 inactivates the same cells of the immune system that protect against viral infection. But tropism for immune cells, as well as the immunosuppression that results, is shared with viruses such as the measles virus, for which an effective vaccine exists (Tatsuo et al., 2000). Perhaps what is critical for HIV-1 persistence is the exact subclass of immune cell targeted by HIV-1 (Chomont et al., 2009) or the selective targeting of HIV-1-specific $CD4^+$ T cells (Douek et al., 2002).

Importantly, like all retroviruses, HIV-1 integrates its genome into host cell chromosomal DNA, where the virus becomes a permanent genetic element in the infected cell and in all daughter cells. This enables it to establish a reservoir of HIV-1-infected cells that persist indefinitely, even in the face of an otherwise effective immune response. In contrast, measles virus cannot integrate, it cannot establish a latent reservoir of infection, and it cannot persist after the measles virus-specific immune response is established.

Histone deacetylase (HDAC) inhibitors activate latent HIV-1 provirus transcription and have been touted as a means to purge the latent reservoir of HIV-1-infected cells (Archin et al., 2012). The underlying idea is that, upon transcriptional activation of latent HIV-1 proviruses, HIV-1 proteins will be synthesized that would render these infected cells detectable by HIV-1-specific cytotoxic T cells. However, upon activation of transcription by the HDAC inhibitors, the HIV-1-infected cells were not killed by autologous, HIV-1-specific CTL unless the CTL were stimulated with cognate antigen prior to provirus reactivation (Shan et al., 2012). These experiments demonstrate that the HIV-1-specific CTL responses in these individuals were somehow defective.

## DC Maturation and Priming of Naive T Cells

Recent research on HIV-1 interaction with dendritic cells (DCs) suggests another hypothesis to explain HIV-1 persistence. DCs are highly heterogeneous, antigen-presenting cells that initiate acquired immune responses by priming naive, antigen-specific T cells (Steinman and Idoyaga, 2010). The nature of the T cell response that ensues, whether tolerance or sterilizing antiviral immunity, is determined by the maturation status of the antigen-presenting DC and the array of signals that the DC provides to the naive, antigen-specific T cell (Figure 1). Among the molecules relevant to T cell priming that DCs elaborate in response to maturation are cell surface MHC Class II, CD80 and CD86, and cytokines such as IL-12 that promote $T_H1$ responses and the cytotoxic T lymphocytes that clear virus-infected cells (Reis e Sousa, 2006; Altfeld et al., 2011).

DCs mature when pattern recognition receptors (PRRs) are stimulated by pathogen-associated molecular patterns (PAMPS) (Akira et al., 2006). Viruses are obligate intracellular parasites, largely dependent upon host cell machinery for replication. They therefore do not generate completely foreign molecules like lipopolysaccharide that distinguish them from the host. Instead, PRRs alert cells to the presence of viruses by detecting more subtle features such as structured replication intermediates, modified nucleic acids, or viral replication complexes in cellular compartments where nucleic acids are not normally found.

Viral nucleic acids can be detected via cell-extrinsic or cell-intrinsic mechanisms (Iwasaki and Medzhitov, 2010). The former include TLR activation after phagocytosis of virus-infected, apoptotic cells. Such extrinsic PRRs are not expressed by all cell types but are generally restricted to antigen-presenting or phagocytic cells, such as dendritic cells. Cell intrinsic mechanisms detect viral nucleic acid within the infected cell, and the PRRs that detect these PAMPs are generally expressed on a broad range of cell types, including fibroblasts. RIG-I and MDA-5 are cell intrinsic cytosolic receptors that detect structural features unique to viral RNA. A cell-intrinsic mechanism also exists for detecting viral DNA in the host cell cytoplasm.

Cytosolic sensors for DNA include IFI16, DDX41, DAI, LSm14A, and AIM2 (Unterholzner et al., 2010; Sharma and Fitzgerald, 2011; Sharma et al., 2011; Zhang et al., 2011; Li et al., 2012; Upton et al., 2012).

Optimal priming of naive T cells to generate potent $CD4^+$ $T_H1$ and $CD8^+$ cytotoxic T cell responses requires that PAMP recognition by PRRs occur within the same DCs that present the antigen, at least under particular experimental conditions (Spörri and Reis e Sousa, 2005; Hou et al., 2008; Kratky et al., 2011). Separation of antigen presentation from PAMP recognition and DC maturation may result in expansion of antigen-specific T cells that lack potent antiviral activity. It is in fact well described that HIV-1-specific T cells express elevated levels of inhibitory molecules such as PD-1, TRAIL, and CTLA-4 (Day et al., 2006; Trautmann et al., 2006; Wherry et al., 2007).

Complicating the assessment of these inhibitory molecules on the HIV-1-specific $CD8^+$ T cells is that these same molecules are upregulated on activated T cells. Coexpression of PD-1 with another negative regulator of T cell activation, CD160, distinguishes a subset of ineffective HIV-1-specific $CD8^+$ T cells from activated T cells (Peretz et al., 2012). While these ineffective, HIV-1-specific, $CD8^+$ T cells are thought to reflect a state of immune exhaustion that arises during chronic infection with HIV-1 (Khaitan and Unutmaz, 2011), similar phenotypes are observed in short-term experiments ex vivo and in humanized mice (Brainard et al., 2009; Lubong Sabado et al., 2009; Che et al., 2010), suggesting that T cell priming to HIV-1 antigens in the absence of optimal DC maturation might contribute to these ineffective, anti-HIV-1 immune responses.

## Innate Immune Detection of Retroviruses

Innate immune detection of viral nucleic acid generally activates type 1 interferon (IFN) and a large number of IFN-stimulated genes with a range of antiviral effector functions. Type 1 IFN also promotes DC maturation and contributes to potent antiviral T cell responses (Longhi et al., 2009). Retroviruses normally do not induce type 1 IFN, perhaps because the level of viral nucleic acid is kept at low level, or because the retrovirus genome is inaccessible to intrinsic PRRs (Fonteneau et al., 2004; Smed-Sörensen et al., 2004; Beignon et al., 2005; Yan et al., 2010; Liberatore and Bieniasz, 2011). This has made it difficult to determine if the innate immune system is capable of detecting retroviruses, and it explains why the study of innate immunity against retroviruses has lagged behind that of other viruses. Unlike other viruses that do induce type 1 IFN, retroviruses are generally believed not to encode specific proteins that block innate immune signaling, although some recent reports suggest that HIV-1 disrupts RIG-I and a downstream transcription factor, interferon regulatory factor (IRF3) (Solis et al., 2011; Doehle et al., 2012a, 2012b). Further, under particular experimental conditions in vitro, aborted HIV-1 reverse transcripts in $CD4^+$ T cells have been shown to activate apoptosis and inflammation in a process that involves caspase-3 and caspase-1 (Doitsh et al., 2010).

Important information concerning innate immune detection of retroviruses has been obtained by studying rare instances where adaptive immune control of retroviral infection has been observed. Specific strains of inbred mice such as I/LnJ and C57BL/6J, for example, are resistant to infection with the murine retroviruses Murine Leukemia Virus (MuLV) and Mouse Mammary Tumor Virus (MMTV) via both humoral and cellular mechanisms. This experimental system offers an opportunity to identify the innate immune PRRs that contribute to these protective, antiretroviral acquired immune responses. The protective antibody responses to both viruses in these mice were TLR7 dependent (Kane et al., 2011b), but induction of protective CTL responses was dependent on a separate, yet to be defined, innate immune detection pathway (Browne and Littman, 2009; Kane et al., 2011b).

The importance of innate immune detection of retroviruses for subsequent acquired immunity was also demonstrated in experiments concerning the influence of the route of infection. When transmitted to pups via ingestion of mother's milk, MMTV acquires a coat of LPS derived from intestinal bacteria (Kane et al., 2011a). Upon engulfment of these virions by the host cells, TLR4 signaling was activated by the virion-associated LPS. The subsequent cascade of signals resulted in production of IL-10, a tolerogenic cytokine that permitted chronic infection. When MMTV was ingested following sterilization of gut bacteria, or when MMTV infection was initiated parenterally, tolerance to the virus was not observed, and chronic infection did not ensue.

## HIV-1 Sensing by DCs

Several types of DCs have been described, though they can be divided into two major groups, the myeloid $CD11c^+$ conventional DC (cDC) and the plasmacytoid DC (pDC) (Steinman and Idoyaga, 2010; Altfeld et al., 2011). The pDC, in contrast to the cDC, constitutively expresses TLR7 and TLR9 (Kadowaki et al., 2001). The pDC endocytoses HIV-1 virions via Env interaction with CD4, and the virion genomic RNA is detected by TLR7 within the endocytic compartment (Fonteneau et al., 2004; Beignon et al., 2005; Smed-Sörensen et al., 2005); this innate immune detection of HIV-1 does not require viral replication and yet results in the production of copious amounts of type 1 IFN. A clear role for the pDC in priming of naive, HIV-1-specific $CD4^+$ T cells has not been established, but the IFN secreted by pDC in response to HIV-1 promotes cDC maturation and NK cell activation (Fonteneau et al., 2004; Romagnani et al., 2005). The complex interplay between NK cells, T cells, and DCs, as well as the direct effects of NK cells on HIV-1-infected cells, has been discussed extensively in a recent review (Altfeld et al., 2011).

The importance of the cDC for priming of naive, anti-HIV-1 T cells is better established than it is for the pDC. HIV-1-pulsed, monocyte-derived DCs, the most common experimental source of human cDCs, prime naive $CD4^+$ and $CD8^+$ T cells in vitro to generate broad, polyfunctional, anti-HIV-1 responses targeting epitopes that mirror those detected after acute infection in vivo (Lubong Sabado et al., 2009). Humanized mice challenged with HIV-1 elaborate similar HIV-1-specific $CD4^+$ and $CD8^+$ T cell responses (Brainard et al., 2009). But, as has been well described in HIV-1-infected people (Day et al., 2006; Trautmann et al., 2006; Wherry et al., 2007), the HIV-1-specific T cells elicited in these assays express elevated levels of inhibitory molecules such as PD-1, TRAIL, and CTLA-4 that suppress the priming of other naive T cells in a contact-dependent manner (Brainard et al., 2009; Che et al., 2010).

In contrast to pDCs, cDCs challenged with HIV-1 do not make type 1 IFN, and additionally, they do not produce IL-12

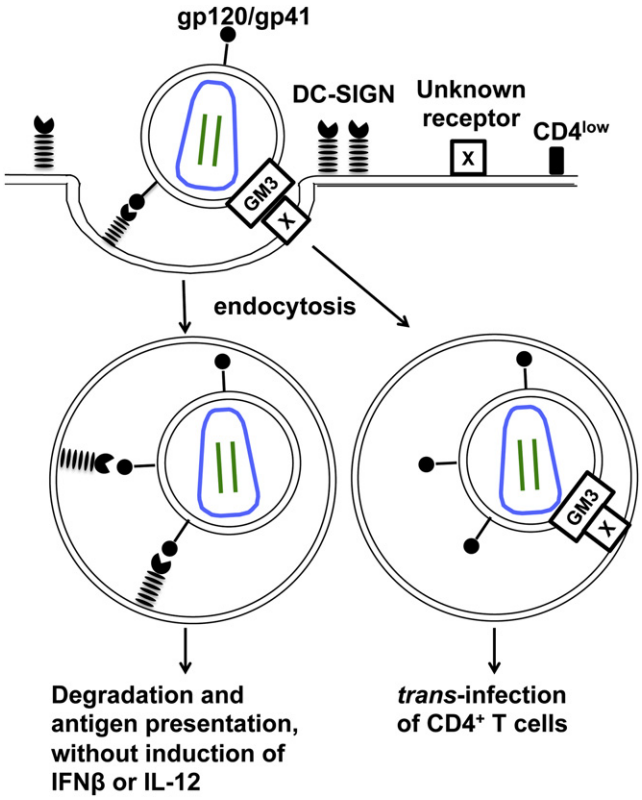


**Figure 2. HIV-1 Virion Interaction with Dendritic Cells Is Dominated by Nonproductive Entry Pathways**

Productive infection requires that HIV-1 virions gain access to the target cell cytoplasm via HIV-1 glycoprotein (gp120/gp41) interacting with host cell receptors (CD4 and either CCR5 or CXCR4). Cell surface levels of CD4 are low on many types of dendritic cells, and HIV-1 virions are endocytosed via interaction with cell surface lectins such as DC-SIGN. Replication of the HIV-1 genomic RNA (green lines) does not occur within the endocytic compartment, and innate immune signaling is not activated within conventional, antigen-presenting DCs. However, the endocytosed virion is degraded, and HIV-1 proteins are therefore efficiently presented in the absence of DC maturation. Alternatively, the glycosphingolipid GM3 in the HIV-1 virion membrane is recognized by an unknown receptor on DCs (designated X). This results in virion capture and transfer to susceptible $CD4^+$ T cells via infectious synapses.

(Fonteneau et al., 2004; Smed-Sörensen et al., 2004; Beignon et al., 2005). The type 1 IFN produced by pDCs in response to challenge with HIV-1 promotes cDC maturation in *trans* (Fonteneau et al., 2004), but as mentioned above, if PAMP recognition and antigen presentation do not occur within the same cell, the critical signals needed for full maturation of cDCs and antiviral immunity may be lacking (Spörri and Reis e Sousa, 2005; Hou et al., 2008; Kratky et al., 2011). These observations demonstrate that cell-intrinsic, innate immune sensing of HIV-1 is insufficient to effect complete cDC maturation. Given the clear importance of the cDC for T cell priming, the discussion that follows below will focus on this DC subtype.

## Nonreplicative Interactions Dominate HIV-1 Entry into DCs

cDCs fail to mature upon HIV-1 exposure probably because the virus cannot infect these cells (Cameron et al., 1992; Smed-Sörensen et al., 2005). Productive infection by HIV-1 requires virion membrane fusion with the target cell membrane, driven by the interaction between HIV-1 gp120/gp41 and the cell surface entry receptors CD4 and CCR5. This results in delivery of the virion core into the cytoplasm where reverse transcription takes place (McDonald et al., 2002). Levels of cell surface CD4 and CCR5 are relatively low on most DC subsets (Caux et al., 1992; Granelli-Piperno et al., 2006), and when HIV-1 encounters DCs, it is more likely to be endocytosed via interaction with DC-SIGN or other C-type lectins (Piguet and Steinman, 2007) (Figure 2). Reverse transcription does not occur within this endocytic compartment, PRRs are not activated, and DCs fail to mature (Granelli-Piperno et al., 2004).

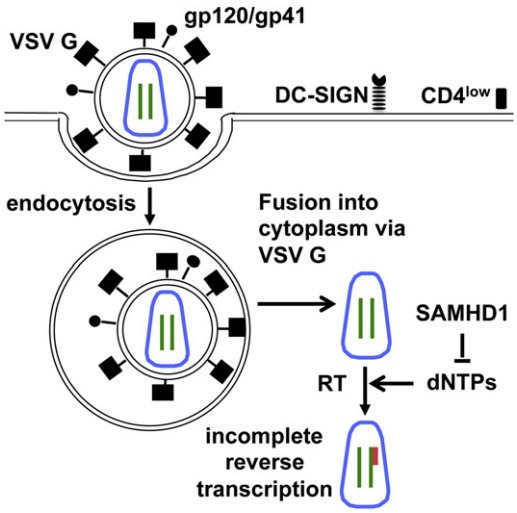


**Figure 3. Bypass of the Block to Productive Entry Reveals a Block to HIV-1 Reverse Transcription in DCs due to SAMHD1**

The block to productive entry can be overcome experimentally by producing HIV-1 virions bearing the vesicular stomatitis virus glycoprotein (VSV G). Once in the dendritic cell cytoplasm, HIV-1 virions fail to reverse transcribe the viral genomic RNA template (green) into viral cDNA (red), because the dNTP concentration is too low to support cDNA synthesis. This block to reverse transcription is due to the dNTP triphosphohydrolase SAMHD1.

HIV-1 virions that are endocytosed by DCs are probably transferred to a lysosomal compartment—and a fraction to proteasomes—and rapidly degraded for processing and presentation in the absence of DC maturation (Moris et al., 2004, 2006). Some virions escape degradation by trafficking to immunologic synapses, from which they are efficiently transferred to highly permissive $CD4^+$ T cells (Pope et al., 1994; McDonald et al., 2003). Avoidance of the degradation pathway and transfer of infectious virions to immunologic synapses is an Env-independent process that requires incorporation of the host cell-derived, glycosphingolipid GM3 into the HIV-1 virion membrane (Izquierdo-Useros et al., 2012; Puryear et al., 2012). The outcome of interaction with the DC C-type lectin and GM3 pathways would be tolerance to HIV-1 and potentiation of HIV-1 spread to highly permissive, activated $CD4^+$ T cells (Figure 2).

Experimentally, HIV-1 virions can be pseudotyped with heterologous viral glycoproteins (Figure 3). This technique can be exploited to redirect HIV-1 transduction to cells that are normally not targeted by HIV-1. Pseudotyping of HIV-1 with the vesicular stomatitis virus glycoprotein (VSV G) drives fusion of HIV-1 virions from endosomes into the cytoplasm and greatly

increases the efficiency with which HIV-1 enters the dendritic cell cytoplasm (Granelli-Piperno et al., 2000). This has become the standard methodology for transducing dendritic cells with HIV-1-based vectors (Berger et al., 2011).

## SAMHD1 Prevents HIV-1 Reverse Transcription in DCs

Although VSV G pseudotyping increases the efficiency with which HIV-1 virions enter DCs, a second block to replication occurs at the level of reverse transcription (Figure 3). The first clue to the nature of this block came from the observation that viruses of the human immunodeficiency virus type 2 (HIV-2) and the simian immunodeficiency SIVsm/SIVmac lineage are more efficient than HIV-1 at transducing DCs and that transduction by $SIV_{MAC}$ vectors required the *vpx* accessory gene (Mangeot et al., 2002). When provided in *trans*, SIV virus-like particles (VLPs) greatly increased the efficiency of HIV-1 reverse transcription and transduction in DCs (Goujon et al., 2006). This increase in HIV-1 infectivity also required the SIV Vpx protein (Goujon et al., 2006) and was mimicked by proteasome inhibitors (Goujon et al., 2007). Vpx was then shown to associate with the CUL4A E3 ubiquitin ligase complex via direct binding to DCAF1 (Sharova et al., 2008; Srivastava et al., 2008; Bergamaschi et al., 2009), and heterokaryon experiments demonstrated the presence of a dominant-acting inhibitor in myeloid cells that was sensitive to Vpx (Sharova et al., 2008). The observation that myeloid cells have 100 times lower dNTP pools than do HIV-1-permissive, activated $CD4^+$ T cells (Diamond et al., 2004; Kennedy et al., 2010) was an additional clue to the mechanism of the block to reverse transcription, since reverse transcription requires dNTPs for synthesis of viral cDNA.

Vpx was then shown to promote the degradation of SAMHD1 (Goldstone et al., 2011; Powell et al., 2011; Lahouassa et al., 2012), a triphosphohydrolase that converts deoxynucleoside triphosphates (dNTPs) to the constituent deoxynucleosides and inorganic triphosphate (Goldstone et al., 2011; Powell et al., 2011; Lahouassa et al., 2012). It was reasonable to hypothesize that, by depleting dNTPs, SAMHD1 would block reverse transcription. Indeed, the kinetics of SAMHD1 degradation that followed Vpx delivery correlated with rising dNTP levels and subsequent increase in HIV-1 reverse transcription (Kim et al., 2012). SAMHD1 knockdown permitted HIV-1 reverse transcription to proceed in myeloid cells in the absence of Vpx (Hrecka et al., 2011; Laguette et al., 2011). Cells from Aicardi-Goutières Syndrome (AGS) patients that bear mutations in SAMHD1 were similarly permissive for HIV-1 in the absence of Vpx. This inflammatory disease will be discussed further below. The antiviral effect of SAMHD1 has also been reported more recently in nondividing, resting $CD4^+$ T cells (Baldauf et al., 2012).

## Consequences of DC Transduction

Efficient HIV-1 transduction of DCs can be achieved by pseudotyping particles with VSV G in the presence of Vpx-bearing VLPs or exogenous nucleosides. One group has reported that DC maturation can occur in response to such high-efficiency transduction by HIV-1 and that DC maturation was associated with increased responsiveness of HIV-1-specific T cell clones (Manel et al., 2010). DC maturation required interaction between the cellular protein cyclophilin A and HIV-1 capsid protein synthesized de novo from the provirus (Manel et al., 2010). Cyclophilin A is a well-characterized HIV-1 capsid-binding protein that regulates HIV-1 infectivity (Sokolskaja and Luban, 2006).

Other groups have failed to detect maturation of DCs after HIV-1 transduction (Fonteneau et al., 2004; Granelli-Piperno et al., 2004, 2006; Beignon et al., 2005; Smed-Sörensen et al., 2005; Pertel et al., 2011a, 2011b), even when transduction was pushed to high levels with Vpx. Perhaps this discrepancy is due to variation in additional host factors that regulate HIV-1 replication in DCs, such as TREX1 (discussed below), or other myeloid-specific, innate immune factors that are yet to be discovered. For example, Vpx rescues HIV-1 transduction of DCs from the antiviral state established by prior treatment with exogenous type 1 IFN via a mechanism that is independent of SAMHD1 and DCAF1-CUL4A (Pertel et al., 2011b).

Infection with HIV-2, a virus that expresses Vpx, is less likely to cause AIDS than HIV-1 (de Silva et al., 2008). Perhaps by permitting HIV-2 to complete reverse transcription within DCs, Vpx enables cell-intrinsic PRRs within these cells to sense HIV-2. Signaling from the PRRs would direct the antigen-presenting DCs to mature and thus render acquired immune responses against HIV-2 more effective. Consistent with this idea, polyfunctional, virus-specific T cell responses are more commonly observed with HIV-2 than with HIV-1 (Duvall et al., 2008). Further evidence in support of this model comes from experiments in which an HIV-1 provirus was modified to express and package SIV Vpx; the resulting virus infected myeloid cells more efficiently than did the parental HIV-1 virus (Sunseri et al., 2011). Additionally, type 1 IFN was detected in these spreading infections (Sunseri et al., 2011), something that was not seen with wild-type HIV-1.

The correlation between Vpx and immune control is not perfect, in that $SIV_{MAC}239$, another virus that encodes Vpx, is highly pathogenic in a nonnative host monkey species. However, $SIV_{SM}$, the naturally occurring virus from which $SIV_{MAC}239$ was derived, is not pathogenic in its native host (Chahroudi et al., 2012).

## TREX1 Conceals HIV-1 cDNA from Detection by the Innate Immune System

The presence of DNA in the cytoplasm of DCs and other cell types, whether from a transfected plasmid, synthetic dsDNA, intracellular bacteria, or infection with a DNA virus, activates type 1 IFN (Sharma and Fitzgerald, 2011). The nuclease TREX1 was identified in a biochemical screen intended to determine the identity of the PRR that activates type 1 IFN in response to DNA within the cytoplasm of macrophages (Stetson et al., 2008). However, in the follow-up functional test, TREX1 disruption did not block type 1 IFN induction by DNA, demonstrating that TREX1 was not the sought-after PRR. Paradoxically, TREX1-deficient animals had elevated type 1 IFN and presented with inflammatory myocarditis (Stetson et al., 2008). Additionally, cDNA from endogenous retroelements was increased in TREX1-deficient cells, consistent with a role for TREX1 in suppressing retroviral cDNA accumulation and blocking cell-intrinsic detection of DNA (Stetson et al., 2008). Amazingly, reverse transcriptase inhibitors that prevent synthesis of retroviral cDNA limited the inflammatory disease in TREX1 null mice (Beck-Engeser et al., 2011).

Subsequently it was reported that when TREX1 is disrupted, challenge with HIV-1 resulted in elevated levels of HIV-1 cDNA and type 1 IFN induction (Yan et al., 2010). This study

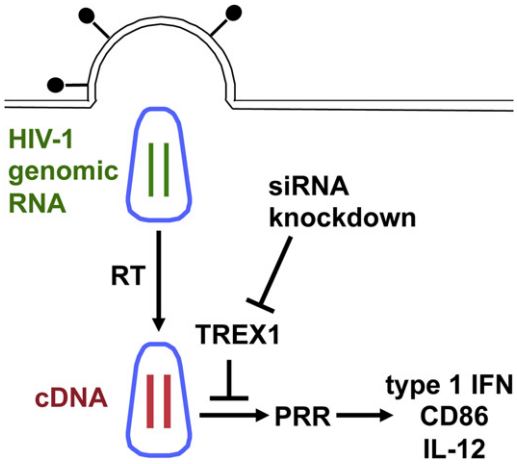


**Figure 4. The Human Exonuclease TREX1 Limits the Level of HIV-1 cDNA**

HIV-1 reverse transcriptase generates a cDNA (red lines) from the HIV-1 genomic RNA (green) in the target cell cytoplasm. Normally, the HIV-1 cDNA is not detected by pattern recognition receptors (PRRs) that recognize DNA in the cytoplasm. When the host cell TREX1 nuclease is disrupted, either by mutation or by siRNA, HIV-1 cDNA accumulates to supranormal levels and activates type 1 IFN. Though several candidate receptors exist (e.g., IFI16), the cell-intrinsic PRR that recognizes HIV-1 cDNA under these conditions has not been identified.

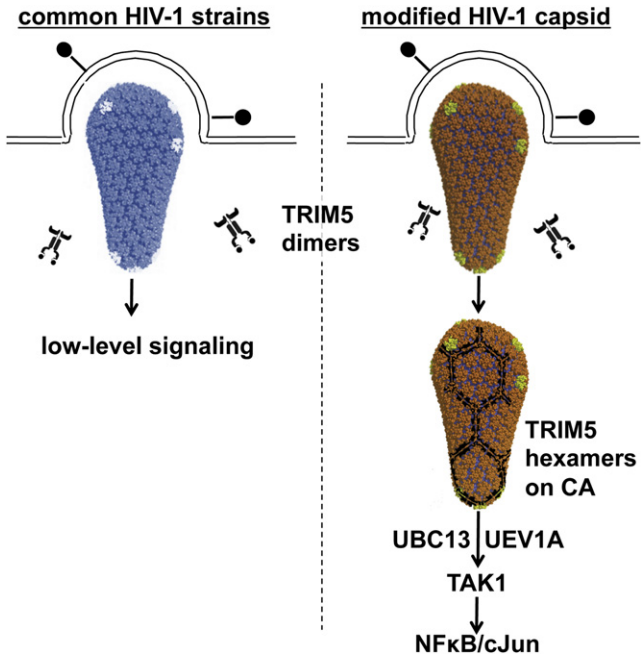


**Figure 5. TRIM5 Is a Pattern Recognition Receptor Specific for the Retrovirus Capsid Lattice**

The cytoplasmic E3 ubiquitin ligase TRIM5 exists as a dimer in the cytoplasm where it signals weakly or not at all. Upon infection with a retrovirus, if avidity for the capsid lattice is great enough, TRIM5 multimerizes to form a complementary lattice (Ganser-Pornillos et al., 2011), and E3 ubiquitin ligase activity is increased (Pertel et al., 2011a). In complex with the heterodimeric E2 (UBC13/UEV1A), TRIM5 synthesizes unattached K63-linked ubiquitin chains that activate the TAK1 kinase complex and innate immune signaling (Pertel et al., 2011a). Most HIV-1 strains (blue capsid) are only weakly detected by the human TRIM5 ortholog. HIV-1 capsid variants that are better recognized by human TRIM5 (orange capsid) would increase innate immune signaling and might make better immunogens.

demonstrated that HIV-1 cDNA can be sensed by the innate immune system via an intrinsic mechanism. However, the cDNA resulting from HIV-1 reverse transcription does not normally accumulate to sufficient levels to be detected unless TREX1 is disrupted (Figure 4). The PRR responsible for detecting HIV-1 cDNA under these conditions has not been determined, although the cytosolic viral DNA sensor IFI16 is a possible candidate (Unterholzner et al., 2010; Sharma et al., 2011).

Mutations in the gene encoding TREX1, as well as in SAMHD1, the HIV-1 restriction factor specific to dendritic cells, macrophages, and nondividing T cells, are associated with Aicardi-Goutières Syndrome (AGS). This rare, lethal, inflammatory syndrome is characterized by elevated type 1 IFN (Crow and Rehwinkel, 2009; Rice et al., 2009). AGS-associated mutations are also found in genes encoding each of the three proteins that constitute the enzyme RNASEH2 (Crow et al., 2006). This enzyme cleaves ribonucleotides within RNA-DNA duplexes like those found in the intermediate products of reverse transcription. Whether RNASEH2 influences innate immune recognition of HIV-1 in DCs remains to be determined. The current model for AGS pathophysiology, whether due to mutations in TREX1, SAMHD1, or RNASEH2, is that inflammation results from activation of cytosolic PRRs in response to the increased levels of endogenous reverse transcripts or reaction intermediates that accumulate when either of these factors is mutated. By demonstrating that the human innate immune system is indeed capable of detecting HIV-1, the AGS factors offer a new paradigm in AIDS vaccine research.

## TRIM5, CA-Specific Restriction, and Innate Immune Signaling in DCs

TRIM5 was identified in expression screens seeking the factor responsible for an anti-HIV-1 activity in diverse cell types from macaques and owl monkeys (Sayah et al., 2004; Stremlau et al., 2004). The TRIM5 orthologs from these two nonhuman primates were then shown to potently block HIV-1 transduction (10- to 100-fold). The viral determinant for sensitivity to TRIM5 restriction is the capsid protein (CA), but TRIM5 binds to the capsid lattice, not free capsid protein (Grütter and Luban, 2012) (Figure 5), and binding induces TRIM5 to form a complementary lattice (Ganser-Pornillos et al., 2011). Because the TRIM5-CA interaction is so complicated, a robust binding assay has not yet been developed, and conclusions about binding strength are extrapolated from restriction activity assays or nonquantitative, particulate association assays (Stremlau et al., 2006).

Phylogenetic comparisons have shown that the sequences encoding the capsid binding domain of TRIM5 are evolving at rates faster than any coding sequence in the primate genome (Sawyer et al., 2005). It has been suggested that this positive selection is driven by challenges in the remote past from pathogenic retroviruses. The human TRIM5 ortholog weakly inhibits lab strains of HIV-1, on the order of 2-fold, in quantitative, single-cycle infectivity assays (Sokolskaja et al., 2006; Battivelli et al., 2010, 2011). The weak inhibition of HIV-1 by human TRIM5 is remedied by replacing a few capsid binding residues in the human ortholog with those from the macaque (Stremlau

et al., 2005). It is possible that human TRIM5 is an active restriction factor in vivo and that it just does not recognize lab strains of HIV-1 very well. One group has recently cloned HIV-1 sequences directly from infected people and found impressive variation in TRIM5 sensitivity. Some HIV-1 isolates were ten times more sensitive than the lab strain standard (Battivelli et al., 2010, 2011). Interestingly, the variant amino acids in these TRIM5-sensitive strains were located in dominant CTL epitopes within capsid, suggesting that escape from CTL forced the acquisition of increased TRIM5 sensitivity.

TRIM5 promotes innate immune signaling (Tareen and Emerman, 2011; Pertel et al., 2011a) via a mechanism that involves synthesis of unattached K63-linked ubiquitin chains and activation of the TAK1 kinase complex (Pertel et al., 2011a) (Figure 5). This biochemical activity, and correspondingly the innate immune signaling within DCs, was amplified greatly by infection with retroviruses that bear restriction-sensitive capsids (Pertel et al., 2011a). The results demonstrating that TRIM5 acts as a PRR specific for the retrovirus capsid lattice were obtained using select combinations of TRIM5 orthologs with specific retrovirus capsids (Pertel et al., 2011a). For example, human TRIM5 in DCs or macrophages signaled in response to the restricted N-tropic MLV, but not to the isogenic, nonrestricted, B-MLV. Owl monkey TRIM5 signaled in response to restricted HIV-1, but not to the unrestricted SIV.

Induction of cytokines was not detected in human DCs challenged with HIV-1, most probably because the avidity of human TRIM5 for capsid from standard strains of HIV-1 is too weak to activate signaling. For signaling to occur in human dendritic cells in response to HIV-1 capsid, the avidity of the interaction will need to be increased. This might be achieved by using HIV-1 capsid variants with CTL escape mutations that confer greatly increased HIV-1 sensitivity (Battivelli et al., 2010, 2011) (Figure 5). Alternatively, if robust binding assays are developed for measuring the interaction of TRIM5 with soluble capsid lattice components (Zhao et al., 2011), screens might be undertaken to identify small molecules that increase the avidity of the interaction.

## Conclusions and Perspectives

HIV-1 vaccine development continues to present enormous intellectual and technical challenges, demonstrating the ongoing need for fundamental biological research concerning pathogen immunity. Several well-characterized properties of HIV-1 explain why vaccine development is so difficult. Additionally, many observations suggest that HIV-1 detection by PRRs within DCs is insufficient to mature antigen-presenting DCs, with the result that naive, HIV-1-specific T cells are not primed for optimal anti-HIV-1 immunity. Progress toward a vaccine will likely require that we understand how conditions can be modified such that HIV-1 is better detected by the innate immune system. Heightened detection of HIV-1 by DCs would result in qualitative improvement in T cell priming, increased survival and effector function of anti-HIV-1 $CD8^+$ T cells, and the requisite affinity maturation for generation of broadly neutralizing anti-HIV-1 antibodies.

Optimal T cell priming likely requires HIV-1 replication within DCs and the resulting activation of intrinsic PRRs. A single block to HIV-1 replication in DCs would be sufficient to preclude a potent acquired immune response. The large number of recently identified blocks to productive HIV-1 infection within these cells, as discussed above, is therefore quite impressive. Expression of DC-SIGN and low levels of CD4 on the surface of DCs, along with GM3-glycosphingolipid within the HIV-1 virion membrane, divert HIV-1 away from productive entry pathways. Reverse transcription is prevented by the nucleotidase SAMHD1. If HIV-1 cDNA is synthesized, the host nuclease TREX1 prevents the cDNA from accumulating to levels that are detectable by cytoplasmic PRRs. Innate immune signaling pathways are not activated by human TRIM5 unless CTL forces the HIV-1 capsid to mutate such that the avidity of TRIM5 for the capsid protein lattice is increased.

This fundamental biological information, along with technical advances in molecular biology, offers experimental approaches to improve DC maturation in response to HIV-1 infection. Nonproductive entry pathways can be overcome by pseudotyping HIV-1 virions with VSV G. dNTP levels necessary for reverse transcription can be achieved by knockdown of SAMHD1, delivery of Vpx, or deoxynucleosides. TREX1 knockdown permits HIV-1 cDNA to accumulate to sufficient levels to activate cytoplasmic PRRs. HIV-1 capsid variants that better engage TRIM5 can potentiate the innate immune signaling elicited by the other measures.

It will be necessary to determine which combination of the interventions described above allows maximal DC maturation and requisite priming of naive anti-HIV-1 T cells. These experiments could be completed in tissue culture using human blood cells or using humanized mouse models and may provide tools and insights that could be translated to immunize humans. For example, autologous DCs treated ex vivo with HIV-1 vectors, immunogens, or drugs might be reinjected as a vaccine. Simpler vaccine formulations would of course be preferable and will hopefully spring from the information these experiments provide.

Going forward, valuable information that might come from these experiments would include the identification of the PRR recognizing HIV-1 cDNA; this PRR would be an appealing cellular target for new adjuvant development. Other than IL-12, what are the specific factors produced by mature, antigen-presenting DCs that optimally prime naive T cells for protection against HIV-1? What is the identity of the glycosphingolipid GM3 receptor in DCs, and would its blockade increase the immunogenicity of HIV-1? Would knockdown of RNASEH2, a gene that is mutated in Aicardi-Goutières syndrome, increase the level of HIV-1 reverse transcription intermediates and activate type 1 IFN, as is seen with TREX1 and HIV-1 cDNA? What is the minimal structure of the retrovirus capsid lattice that is recognized by TRIM5, and could this information be exploited to improve HIV-1 CA detection by the human TRIM5 ortholog? Ultimately, it will be of great interest to know if new vaccines that increase the intrinsic detection of HIV-1 within antigen-presenting DCs reduce the frequency of new HIV-1 infection in populations at risk for HIV-1.

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